Saskatchewan
Agriculture
and Food

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20030446

DEVELOPMENT OF NOVEL ADJUVANTS TO IMPROVE THE EFFICACY OF NEONATAL CALF DIARRHEA VACCINES

Funded by: The Agriculture Development Fund

January 2007

Prepared by: University of Saskatchewan

2007-04627/

Development of Novel Adjuvants to Improve Efficacy of Neonatal Calf Diarrhea Vaccines

ADF Project # 20030446

Final Report

November 30, 2006

by

Drs. George Mutwiri & Jan van den Hurk

Vaccine & Infectious Disease Organization (VIDO)

University of Saskatchewan

120 Veterinary Road

Saskatoon, SK

S7N 5E3

(306) 966-7465

www.vido.org

TABLE OF CONTENTS

	Page
Executive Summary	2
List of Figures	3
Scientific Report	
i) Background	4
ii) Objectives	5
iii) Methods	5
iv) Results	6
v) Conclusions and Recommendations	7
vi) Acknowledgements	8
vii) Literature Cited	8
viii) Administrative and Other Aspects	8
Appendices	9

EXECUTIVE SUMMARY

Vaccination remains the most cost-effective biomedical tool for the control of infectious diseases in livestock. Vaccines based on killed pathogens or subunit antigens are safer but are often ineffective and require addition of adjuvants to improve their efficacy. Currently available vaccine adjuvants have a number of limitations including induction of low level, short duration immunity, and severe tissue reaction at the site of injection. Clearly, there is a great need for safe and effective new adjuvants to reduce the incidence of economically important diseases. The objective of this project was to develop novel vaccine adjuvants/formulations to improve the efficacy of neonatal calf diarrhea vaccines. Rotavirus is a major cause of neonatal calf diarrhea. For this reason, we tested whether a number of new and conventional adjuvants could improve the efficacy of a rotavirus vaccine. Our investigations revealed that combinations of adjuvants were more effective than individual adjuvants in enhance immune responses to the rotavirus vaccine. Since protection against rotaviral diarrhea is dependent on antibody titers, our results suggest that these new adjuvant formulations have the potential to reduce the incidence of neonatal calf diarrhea and increase economic returns to the producer.

LIST OF FIGURES

	Page
Figure 1: Rotavirus P1P5-specific antibody responses in the serum.	6
Figure 2: P1P5 vaccine-specific antibody responses in the serum.	7

SCIENTIFIC REPORT

i) Background

Infectious diseases cause great economic losses to the animal health industry. Newborn calves are highly susceptible to infectious diseases and diarrhea accounts for approximately 5% of calf mortality during the first month of life (**2**). A significant proportion of diarrheal diseases in calves can be attributed to neonatal diarrhea commonly known as "calf scours". In Saskatchewan, calf scours has been implicated in approximately 36% of all calf deaths and losses of up to $1,000 per beef herd (**3**). Calf scours is caused by infection with a combination of rotavirus, coronavirus and *E. coli*.
As with many other infectious diseases, immunization is considered the most cost-effective approach to control calf scours. Calf scours vaccines usually contain a combination of antigens from all the three etiologic agents; VP8* protein of rotavirus from serotypes P1 and P5, S1 and S2 proteins from coronavirus, and *E. coli* K99 pili. These vaccine components have been shown to induce neutralizing antibodies, and in some cases protection against infection (**2**, unpublished observations). The strategy currently used to control calf scours is dam immunization, whereby pregnant cows are vaccinated to elicit protective antibodies that are then transferred to calves through colostrum and milk (passive immunization). It is thought that only antibody present in the intestine is protective, while circulating antibody is not very helpful (**4**). The recent discovery of an IgG receptor in the intestine of mice (**5**) would suggest that circulating antibody would be transported to the intestine, but the implication of this observation in systemic vaccination of cattle is not clear. However, despite immunization, calf scours remains one of the major causes of economic loss to the beef industry. One approach to reduce the incidence of calf scours is to improve the efficacy of existing vaccines with effective adjuvants. Indeed, the efficacy of many commercially available vaccines is dependent on addition of adjuvants, compounds that enhance the immune responses induced by vaccines. However, conventional vaccine adjuvants have a number of limitations including: (i) induction of low level of immunity, hence not protective, (ii) immunity is of short duration, usually requiring many injections, increasing the cost of vaccination, (iii) are not effective in young animals, although this is the period of highest risk of disease, and (iv) cause severe tissue reaction at the site of injection, costing up to $9 per animal slaughtered in Canada (**6**). Thus, there is a great need for safe and effective adjuvants to increase the efficacy of conventional vaccines and reduce the incidence of economically important diseases such as scours.

Research in our laboratory has demonstrated that a class of synthetic polymers (polyphosphazenes) can enhance immune responses to vaccines over 1000-fold compared to conventional adjuvants in mice following a single immunization. These polymers had never been tested in domestic animals. Therefore, the objective of our research was to develop polyphosphazenes as an adjuvant and a delivery system to improve the efficacy of vaccines for neonatal diarrhea in calves. As the research progressed, it became necessary to test other new adjuvant formulations as well.

ii) Objectives

1. Evaluate the potential of polyphosphazenes as an adjuvant for an experimental rotavirus vaccine
2. Identify novel adjuvant formulations that can improve immune responses to a commercial rotavirus vaccine.

iii) Methods

Vaccine production:

For the first study an experimental vaccine was produced at VIDO as follows: The VP8 gene from bovine rotavirus Type P1 was prepared by PCR amplification using the plasmid construct pGH432-P1. The PCR product was ligated into the construct pGH433-P5 in front of the VP8 gene from Type P5 to generate a new construct designated pGH433P1P5. The plasmid was then transformed into an appropriate bacterial host. An inducible promoter, tac, in the pGH433 plasmid allows for overexpression of the chimeric protein P1P5 by induction with IPTG(isopropyl β-D-thiogalactopyranoside). This overproduction of a highly insoluble inclusion body protein can be accomplished in a variety of bacterial strain, including laboratory *E.coli* strains.

The inclusion body was crudely isolated using a protein aggregate protocol and then solubilized before being run through 3 types of chromatographic columns. Two passes through an anion exchange column preceded both a hydrophobic interaction and size exclusion column. The purified P1P5 protein was greater than 90% pure.

For the second study, a commercial vaccine was obtained from a Canadian animal health company that manufactures vaccines against rotavirus diarrhea in calves.

Immunization of animals:

In the first study, a total of twenty calves (n=5 per group) were immunized with two subcutaneous injections of 50 µg of P1P5 vaccine antigens either alone or in combination with 1 mg of the polymer PCPP or 30% emulsigen (commercial adjuvant). The treatment groups were as follows: P1P5, P1P5+PCPP, P1P5+emulsigen and unimmunized control animals were injected with saline (PBSQ). The site of injection was examined visually and palpated during the first 48 following injection. Animals were bled before immunization and at biweekly intervals following immunization and serum was separated and stored at –20°C until used. P1P5-specific antibody responses were assayed in serum by the ELISA technique.

In the second study, a total of 42 calves were immunized with two subcutaneous injections of 100 µg of a commercial vaccine (containing P1P5 antigens) either alone or in the following combinations: P1P5+1 mg PCPP, P1P5+CpG, P1P5+30% emulsigen, (commercial adjuvant), P1P5+30% emulsigen+PCPP, P1P5+30% emulsigen+CpG, or P1P5+PCPP+CpG. The site of injection was examined visually and palpated during the first 48 following injection. Animals were bled before a primary immunization (day 0) and at 7, 14, 28, 35 42 and 48 days after the primary immunization. A secondary immunization was give at day 28. Serum was separated from the blood and stored at –20°C until used. P1P5-specific antibody responses were assayed in serum by the ELISA using a protocol described previously (**2**) with minor modifications.

iv) Results

In the fist study we tested whether polyphosphazenes had adjuvant activity with a rotavirus antigen in cattle and whether it induces obvious tissue reaction at the site of injection. Additionally, we compared the activity of polymers the conventional adjuvant, emulsigen, which is commonly used in many cattle vaccines, but causes severe tissue reaction. We found that addition of emulsigen to the P1P5 vaccine resulted in a substantial increase in P1P5-specific antibody titers in serum. In contrast, there was essentially no increase in P1P5 antibody titers in all the other two experimental groups (P1P5+PCPP and P1P5 alone) when compared to the control unvaccinated group (Figure 1). While emulsigen is known to cause severe tissue reaction, no tissue reaction was seen at the injection site in animals injected with vaccine formulated in PCPP.

Figure 1: Rotavirus P1P5-specific antibody responses in the serum.

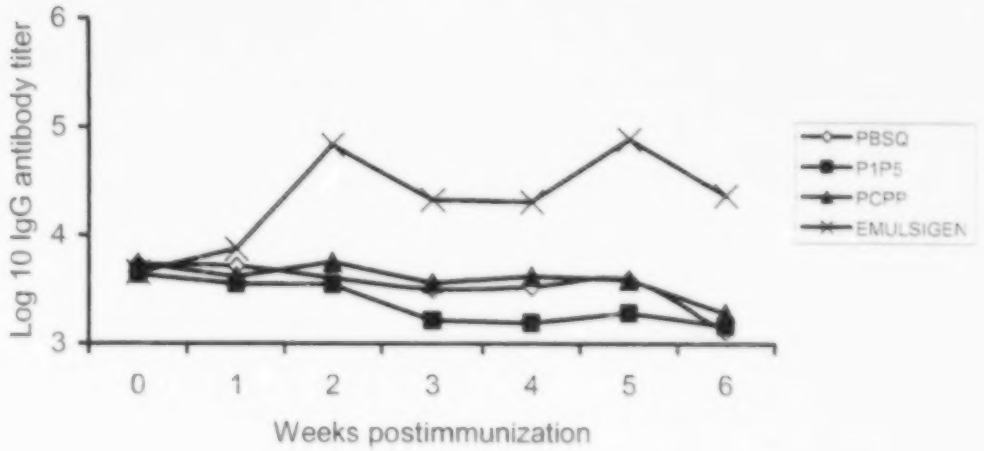

Fig. 1: Calves were immunized with two subcutaneous injections of either P1P5, P1P5+PCPP or P1P5+emulsigen. Control animals were injected with PBSQ. Serum antibody responses were assayed in the serum following immunization. Data points on the graph represent group medians.

Since we did not observe any adjuvant effect with the PCPP and P1P5 vaccine formulation, we made modifications in the experimental design of the second study so as to include other potential novel adjuvants and their combinations.

Our results indicated that all animals had pre-existing anti-P1P5 antibodies. This was expected because all cattle are exposed to rotavirus throughout live. Interestingly, two groups of animals immunized with P1P5+30% emulsigen+CpG, and the other one with P1P5+30% emulsigen+PCPP developed an increase in P1P5-specific antibody responses as early as 7 days after the primary immunization, and continued to increase at day 14 and then declined by day 28. All other groups did not develop any substantial antibody titers to the P1P5 vaccine. Following the secondary immunization, the antibody titers in

animals immunized with either P1P5+30% emulsigen+CpG, or with P1P5+30% emulsigen+PCPP peaked at day 35. In addition to these two groups, the group immunized P1P5+30% emulsigen showed a modest increase in IgG titers but this declined rapidly. None of the other groups showed any substantial increase in antibody titers above background levels, even after the two immunizations.

Figure 2: P1P5 vaccine-specific antibody responses in the serum.

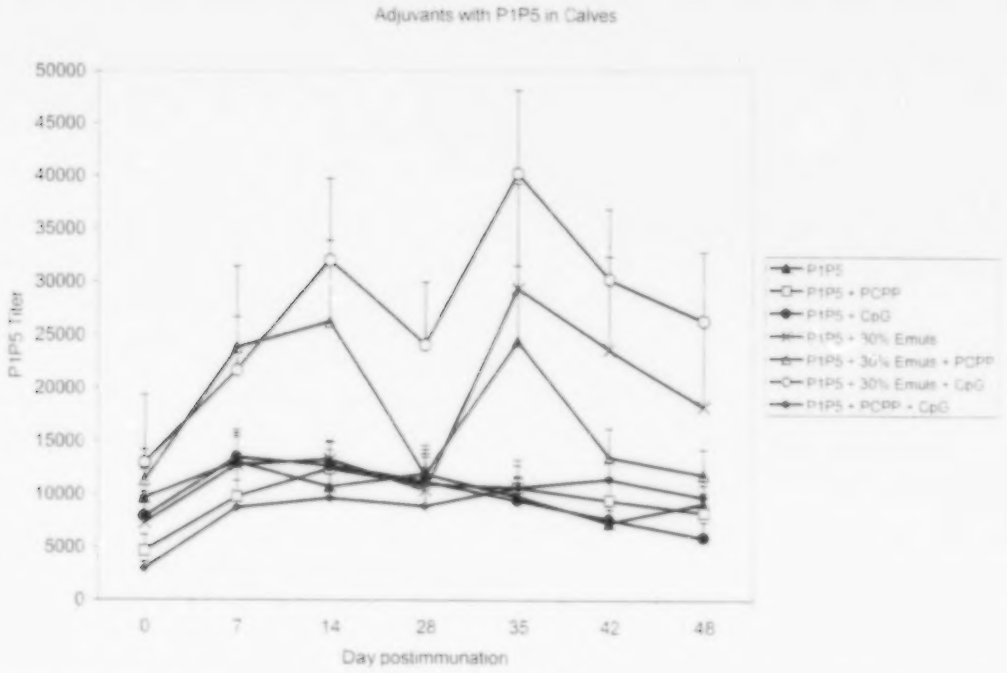

Fig. 2: Calves were immunized with two subcutaneous injections of either a commercial vaccine alone, or the vaccine plus various adjuvant combinations as described in the methods. Serum antibody responses were assayed in the serum following immunization. Data points on the graph represent group means±SE.

v) Conclusions and Recommendations

Using a commercial vaccine for rotavirus diarrhea, we have identified at least two adjuvant combinations that induce good antibody responses even after a single immunization. In addition to being higher, the antibody responses occurred earlier than those induced by the conventional adjuvant. Our recommendation would be that these adjuvant combinations be evaluated in large scale animal clinical trials, since data from such studies would attract a commercial company with resources for commercialization.

(vi) Acknowledgements

Foremost, we would like to thank ADF for funding our research. We have also acknowledged in all presentations where these results were discussed (VIDO seminars and program meetings). Similarly, we plan to acknowledge ADF when we publish these results in a refereed journal (manuscript in preparation).

vii) Literature Cited

1. Dodet et al. 1997 Trends in Microbiology 5:176.
2. Lee, J. et al. 1995. J. Gen. Virol. 76: 2477.
3. Acres, S. & Radostits, O. VIDO Fact Sheet #1.
4. Snordgrass, D.R. et al. 1986 Infect. Immun. 28:344.
5. Lencer et al 2002. J. Exp. Med. 196:303.
6. Van Donkersgoed, J. et al. 1997. Can. Vet. J. 38, 767.
7. Griebel and Hein 1996. Immunology Today 17:30
8. Mutwiri, G.K. et al. 2000. Vaccine. 19, 1284.
9. Mebus, et al. 1973. J.A.V.M.A. 163:880.
10. Elderidge, J.H. et al. 1990. J. Control. Release. 11: 205.
11. Kim, B. et al. 2002. J. Cont. Release. 85:191.
12. Mutwiri, G.K. et al 2002. Vet. Immunol. Immunopathol. 87:269.
13. Periwal, S.B. et al. 1997. J. Virol. 71: 2844.
14. Daar, A.S. et al. 2002. Nat. Genet. 32:229.

viii) Administrative and Other Aspects

These results were presented at VIDO research seminars, and at various VIDO program meetings.

APPENDICES

Not applicable.